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Copper	*Phaeodactylum tricornutum*	
Heavy metals	Sediments	

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

This report describes the results of an algal assay used in studying the toxicity of various soil and sediment samples from the Trident base, located on Hood Canal, in Washington state. Analyses for 24 elements were made on the samples, and analyses for Pb, Cd, Cu, and Zn were made on the algal cells cultured in nutrient-enriched extracts of the samples.

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Some of the samples were toxic according to the algal assay, but there was almost no correlation between the concentrations of Pb, Cu, and Zn in the algal cells and those in the test samples; there were no data on the Cd content of the soil and sediment samples. On the basis of these experiments it is concluded that the Standard Elutriate Test, which is based on chemical analyses of sediment elutriates, has limited value and that more meaningful data would be realized from a bioassay.

CONTENTS

LACK OF CORRELATION BETWEEN THE COMPOSITION OF SEDIMENTS AND THEIR TOXICITY TO ALGAE

INTRODUCTION

The Navy must maintain channels sufficiently deep for the safe passage of its ships, and therefore must engage in dredging operations. Current environmental pollution constraints require knowledge of spoils toxicity prior to their disposal. The ultimate costs of disposal might depend on the toxicity, for if a sediment is shown to be innocuous it might be dumped at an inshore site, whereas a toxic sediment might require hauling to a deep ocean site for disposal.

The present criterion for toxicity is the Standard Elutriate Test, which was devised jointly by the Corps of Engineers and the Environmental Protection Agency [1]: A sediment is classified as a pollutant if the concentration of a specific constituent in the overlying water is increased by as much as 50% upon shaking with the sediment. This chemical approach has several pitfalls:

1. Analyses must be made for each suspected contaminant.

2. The concentrations of heavy metals, as determined by the analyses, may not be interpretable in terms of toxicity because of the variety of chemical species in which they may exist.

3. The combination of several pollutants may be more toxic, or less, than their single effects.

Furthermore, it is possible that the elutriation will often result in the scavenging of heavy metals by the sediment rather than the release of ions from the sediment to the water [2]. For these reasons the chemical approach to the problem of sediment toxicities is suspect, and more useful information should be obtained by a toxicity test involving living organisms. Prior experience proved that algae can be grown reproducibly [3] and therefore they should be considered for use in such an assay; furthermore, their role as the primary producers of food and oxygen in the sea attests to their importance.

Various assays based on algal growth are described in the literature [4-10]. Several of these might be adapted to studies of sediment toxicities but are lacking in one aspect deemed desirable, viz, a means of measuring growth continuously to distinguish temporary inhibitions, after which growth might be normal, from inhibitions of a permanent nature. This problem was approached by developing a procedure in which the CO_2 uptake of algae was measured hourly by the automatic monitoring of pH; previous investigations [11,12] concerning the pH changes of growing algal cultures formed the basis of this new method. In addition to the estimation of CO_2 uptake, measurements of

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fluorescence were also made, which provided an indication of the chlorophyll present in the algal cells.

The algal toxicity test was included in a study of soil and sediment samples from the developing Trident submarine base at the Bangor Annex, Wash., to determine its pertinence. This included samples from the Hood Canal, where continual monitoring of salinity, pH, and dissolved oxygen is performed, and from other areas in the 7800-acre site. Among these were samples from culverts, dump sites, and sediments from freshwater lakes, all of which were identified in Fig. 1 and the following list. Analyses for 24 elements were made by x-ray fluorescence (Analex Inc., Anaheim, Calif.).

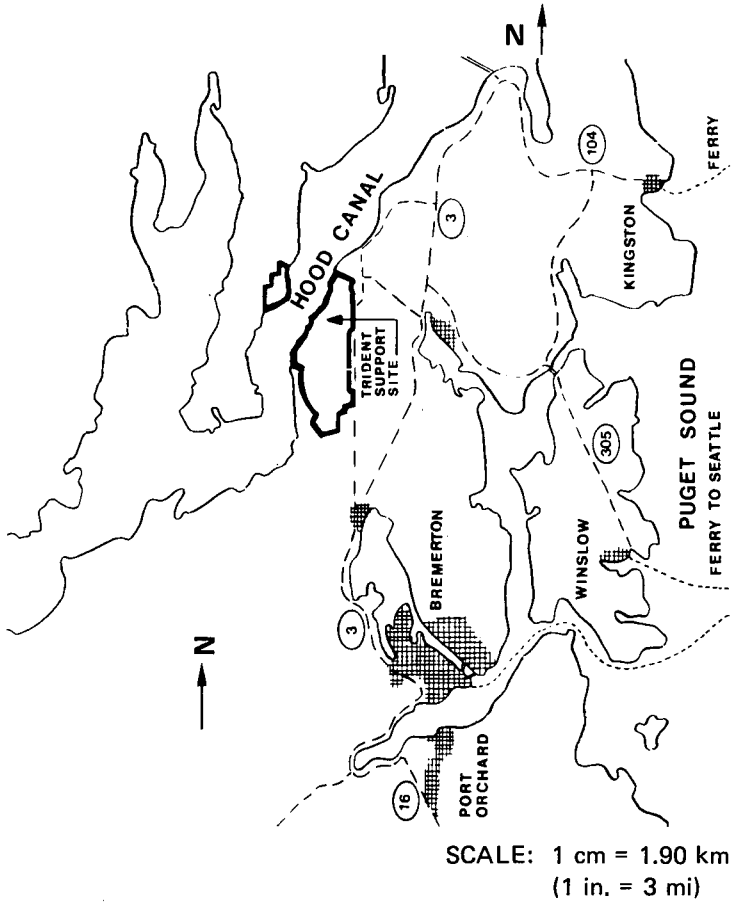

Fig. 1(a) — Location of Trident support site

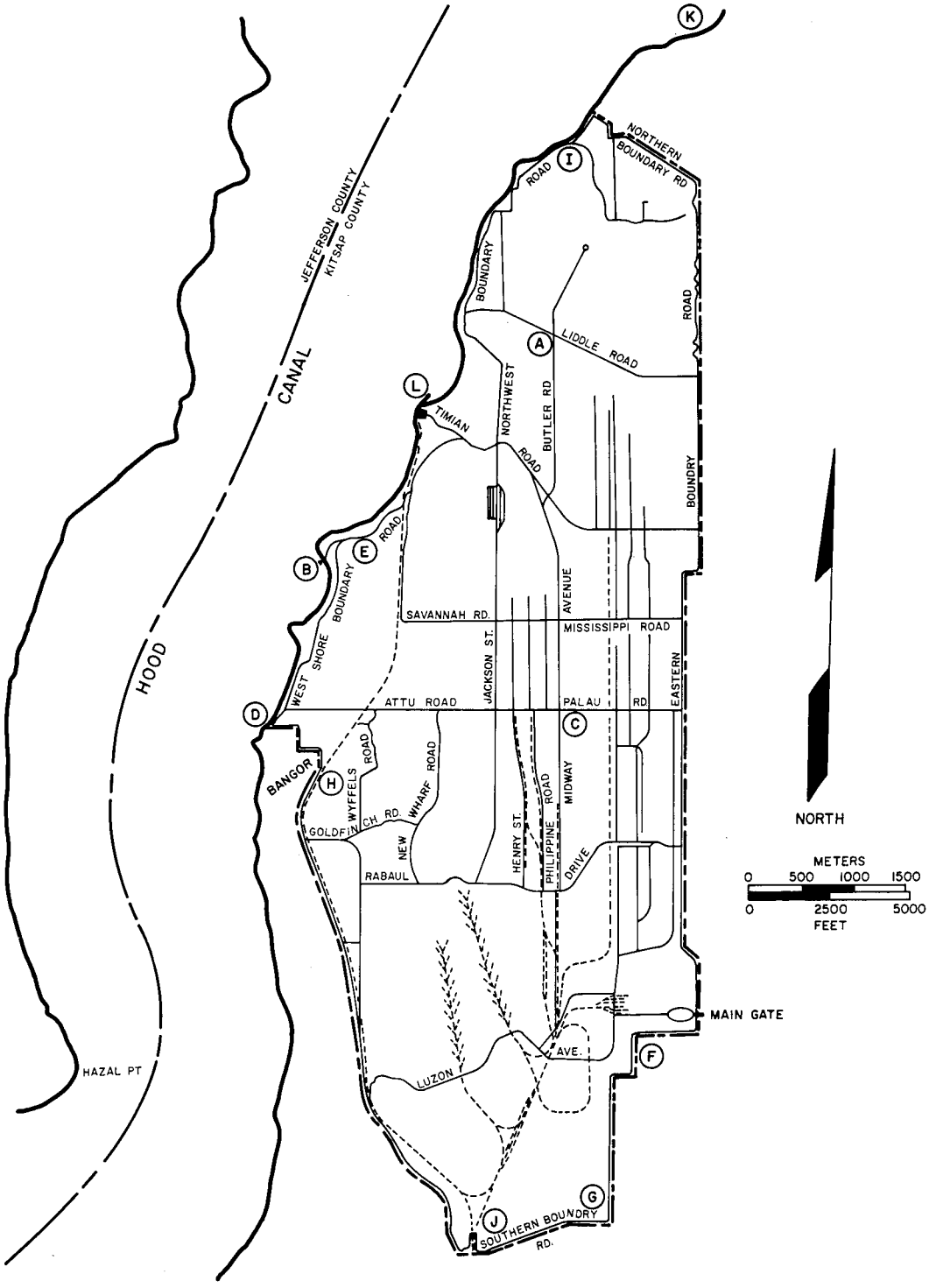

Fig. 1(b) — Location of sediment sampling points

Sediment: Location and Factors Affecting Sediment and Soil Samples

A On high ground; had been a dumping site for Otto fuel. The sample had a greasy feel and a slight greenish tinge. Surrounding vegetation seemed to be healthy and unaffected by previous exposure to the Otto fuel.

B From the Hood Canal at the Keyport-Bangor (KB) wharf, which is used for mooring small ships of approximately 30-m (100-ft) length.

C On high ground; formerly used as a dump site for RDX, TNT, and perhaps sources of heavy metals.

D From the Hood Canal at the site of an old pier that at one time served ships.

E Sediment from Devil's Hole, a shallow freshwater lake.

F A stream bed that receives some runoff water from a parking lot, a sewage plant, and an industrial area.

G From a stream bed in which water flow is often at a high rate; source water is from springs and is uncontaminated.

H From a shallow stream bed that contains clear, fresh water.

I Sediment from Cattail Lake, a manmade freshwater lake about 3 m (10 ft) deep.

J Soil sample from pristine area (control).

K Sediment from Hood Canal in front of private property, about 0.8 km (1/2 mi) north of Navy boundary. A boat house is located here, as is a water quality-control station.

L Sediment from Hood Canal at Marginal Wharf where large ships and submarines are berthed.

The purposes of this study were to determine the toxicity of the samples by the algal assay and to estimate the relevance of the Standard Elutriate Test by comparing the concentrations of several metals (Pb, Cu, Cd, and Zn) in the sediments and in the algal cells harvested from the cultures made with the elutriates. Though no data on the Cd content of the sediments were available, it was felt that the ecological importance of this element justified its inclusion in the study of algal cells.

MATERIALS AND METHODS

Sediments

Twelve of the sediments were from the Bangor Annex, and three were from coastal waters in California. Chemical analyses for those from Bangor Annex are shown in Table 1.

Table 1—Composition of Sediments

Code	Description	Moisture Content (%)	Fraction Dry Weight Through 1.6-mm (1/16-in.) Sieve (%)	Concentrations (ppm)																							
				Al	Si	S	Cl	K	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	As	Se	Br	Rb	Sr	Ba	Hg	Pb
A	Green; greasy	27.6	86.9	24 000	270 000	<1000	<400	9 300	23 000	2600	<330	230	5400	36 000	<90	54	160	290	20	<19	<4	<3	200	390	<900	<4	3700
B	Pebbles; sand; cracked shells	16.3	67.4	29 000	270 000	<1000	3 400	8 900	21 000	2300	<260	200	600	50 000	<80	45	340	1300	<8	600	<2	19	26	270	<800	<4	429
C	Small granules; few pebbles	13.7	71.4	28 000	230 000	<1000	<400	6 500	18 000	2800	<180	210	550	25 000	<60	67	52	52	11	6	<2	3	21	310	<600	<4	11
D	Very fine; no pebbles	54.1	86.3	37 000	200 000	<1000	<400	12 000	22 000	4800	<380	260	1100	46 000	<90	83	72	110	17	<3	<2	4	35	370	<1300	<4	17
E	Very fine; few pebbles	79.5	94.4	30 000	240 000	7600	<400	8 200	16 000	3900	<340	230	770	37 000	<80	74	89	145	12	9	<2	27	31	190	<1100	<4	28
F	Dark gray sand	21.6	96.7	34 000	330 000	<1000	<400	9 000	18 000	2000	<200	75	490	21 000	<50	48	89	70	11	10	<2	<2	21	330	<680	<3	28
G	Sand; small red pebbles	20.1	76.7	34 000	300 000	<1000	<400	8 300	15 500	2100	<200	110	730	26 000	<80	59	53	47	10	5	<2	2	30	295	<680	<3	33
H	Mostly sand; some clay	24.3	97.7	36 000	280 000	<1000	<400	9 600	19 000	1900	<160	120	480	19 000	<60	47	54	67	14	<3	<2	3	28	390	<550	<3	18
I	Very fine clay	78.6	86.8	39 000	210 000	<1000	<400	9 800	18 000	4700	<260	140	1100	54 000	<110	160	91	120	13	29	<2	15	39	260	<880	<4	15
J	Large particles; shells	29.8	27.1	14 000	160 000	4500	12 000	8 500	142 000	1400	<90	40	250	17 000	<60	27	94	140	5	<4	<2	90	18	940	<290	<5	63
K	Gray and black sand	26.1	99.2	30 000	250 000	1700	9600	9 400	18 000	1700	<200	75	340	18 000	<60	31	18	40	13	11	<2	54	34	380	<660	<3	7
L	Large particles; shells	48.6	14.5	21 000	160 000	5300	31 000	10 000	87 000	2500	<110	26	370	33 000	<70	38	170	410	7	25	<2	260	31	700	<360	5	124

Elutriation Procedure

A 300-ml quantity of sediment was mixed with 2000 ml of *Instant Ocean®*, which had been aerated previously with 3% CO_2 in air to reduce its pH to approximately 8.1. After 30 min of stirring, the sediment was allowed to settle (also 30 min), was centrifuged, and the supernatant was filtered through a 0.45-μm Millipore® filter. A 500-ml sample of this particle-free elutriate was acidified and reserved for analyses for Pb, Cu, Cd, and Zn. The remaining elutriate was used in the preparation of the test suspensions.

Test Suspensions

Two concentrations of elutriate were included in each experiment, each containing the same inoculum of cells and the same concentration of added nutrients. The purpose was to aid in distinguishing the growth promotion, fostered by nutrients in the elutriate, from the inhibition caused by heavy metals or other pollutants. Also included in each test was a suspension containing 0.01 mg Hg/l as a reference toxicant. The suspensions were prepared according to the following procedure.

1. A 3000-ml quantity of a dilute culture medium (1:12) was prepared by adding 250 ml of freshly prepared Guillard and Ryther (G&R) medium [13] to 2750 ml Instant Ocean® (pH 8.1). The slight acidity of the G&R medium lowered the pH to approximately 8.0. The G&R medium prepared for these experiments was made with 3.5% NaCl rather than with Instant Ocean®.

2. Triplicate samples of controls and a single sample containing 0.01 mg Hg/l were prepared from the solution described in item 1 of this listing. This was done by adding the required volume of algal cells to the 3000 ml to give a 15-ppm (by volume) suspension, which contained approximately 0.02 μg/ml of chlorophyll. A 500-ml sample of this suspension was set aside for titration (see the section "Growth Conditions and Measurements"), three 500 ml portions were used as controls, and one 500 ml sample was made up to 0.01 mg by adding 0.05 ml of a solution freshly made from a 1:10 dilution of a stock solution containing 1.353 kg/m^3 of $HgCl_2$.

3. Table 2 gives the composition of the suspensions containing the two concentrations of elutriates.

Table 2—Compositions of Suspensions Used in Experiments

Suspension	Volume of Elutriate (cm^3)	Volume of Instant Ocean (cm^3)	Volume of G&R Medium (cm^3)	Volume of Cell Slurry
Concentrated elutriate	917	0	83	Amount required to give 15 ppm
Half-concentrated elutriate	458	458	83	Amount required to give 15 ppm

Each of the suspensions was divided into two 500 ml portions, one to be used in the growth experiment and one to be titrated with standard 0.5 *N* NaOH.

Organism

Phaeodactylum tricornutum was grown as a stock culture (40 ml) in 125-ml Erlenmeyer flasks in the G&R medium. Cultures between two and three weeks old were centrifuged and resuspended in 3.5% NaCl three times to remove excess nutrients. The cells were made into a slurry in the NaCl solution, and their packed cell volume was determined with a Bauer and Schenk centrifuge tube. The volume of slurry required to make a 15-ppm inoculum was then added to the test solutions.

Growth Conditions and Measurements

Six 500-ml test suspensions were placed in a lighted aquarium; these consisted of triplicate controls, one suspension containing 0.01 mg Hg/l as a reference toxicant, one with the concentrated elutriate, and one with the elutriate diluted by half with Instant Ocean®. The temperature of the bath was maintained at 23.0° ± 0.1°C by a coil that was refrigerated continuously and by a 750-W heater operated on demand from a thermoregulator. Each flask contained a magnetic stirring bar and was tightly stoppered; stirring was provided by a unit containing six magnetic rotors. The rubber stopper in each flask was bored to accommodate an electrode (Arthur H. Thomas Model 4094-S10, Ag/AgCl reference, with glass sheath removed) that extended several centimeters into the suspension. The electrodes were connected to an Orion pH meter through an automatic switch that interrogated each electrode hourly over the 48-hr test period, and the pH values were recorded by a digital printer. (The meter, automatic switch, and digital printer are the products of Orion Research, Inc.) The electrodes were conditioned before use by soaking overnight, while illuminated, in a 1:12 culture medium of the same type as used in the tests. Lighting was provided by eight 100-W lamps, operated at 75 V, on each side of the aquarium. The light intensity was 5400 lm/m^2 (500 foot candles). The equipment is shown in Fig. 2.

CO_2 uptake values were deduced from the pH measurements by reference to the titration curve for each suspension. The pH rise resulting from the addition of a milliequivalent of NaOH was assumed to be the same as that brought about by the absorption of a milliequivalent of CO_2 by the algal cells.

Increases in algal cells at the end of 48 hr of incubation were calculated from the fluorescence data and from a standard curve relating cell number to fluorescence. Because some of the sediment elutriates contained fluorescent materials, a correction factor was sometimes required; any fluorescence at the beginning of the experiment that could not be accounted for by the presence of the algal cells was subtracted from the final fluorescence value.

The weight of the algal cells produced by a control culture and by each of the elutriates was determined by filtering through 0.45-μm Millipore® filters. These filters had been impregnated previously with a 1:12 culture medium and dried, to minimize the

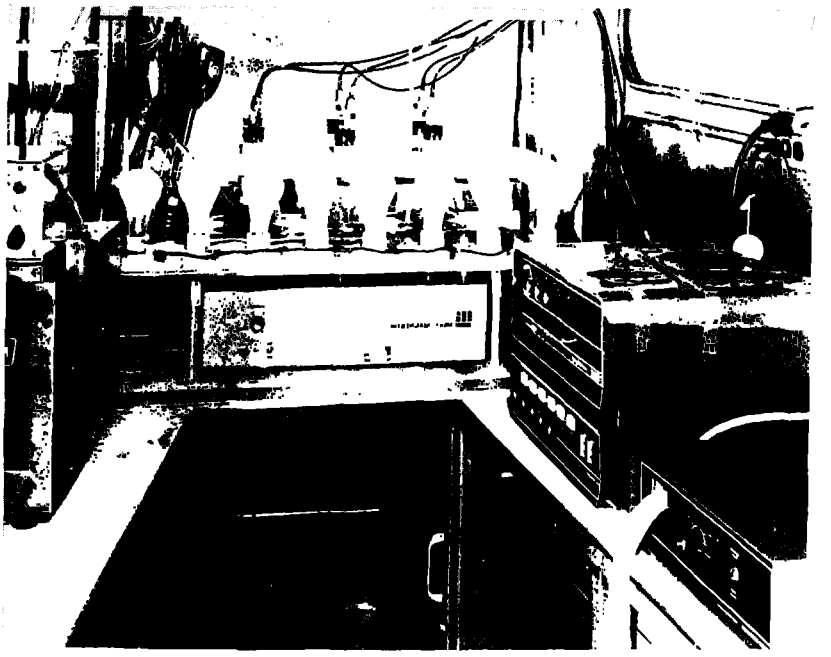

Fig. 2—Algal culture apparatus, with pH-measuring equipment used

effect of adsorbed ions from the algal suspensions. This procedure provided a reasonable estimate of the weight of cells, but in some instances a slightly gelatinous precipitate formed, perhaps Mn or Fe hydroxides, which also was included in the weight. Preliminary experiments had indicated that no precipitate formed in the control suspensions if the pH did not rise above 9.4, but the suspensions grown in certain sediment elutriates gave evidence of precipitation at pH values lower than that.

Analyses for Lead, Cadmium, Copper, and Zinc

The filter papers containing the cells were dissolved in 25 ml of concentrated HNO_3 and analyzed by atomic absorption spectrophotometry [14] after all the growth experiments had been completed, as were the acidified 500-ml elutriates. Also reserved for analyses were several Millipore® filters that were impregnated with one of the 1:12 culture media. These were intended to serve as controls for the adsorption of the trace metals in question to be subtracted from the analyses of the filters containing the cells.

RESULTS

The data are summarized as follows: Table 1 contains analyses for 24 elements in each sediment as determined by x-ray fluorescence; Table 3 lists the concentrations of Pb, Cd, Cu, and Zn in the elutriates of the Bangor samples; Table 4 summarizes the analyses of the cells for these same four metals after two days of growth; Table 5 shows

the growth of the test cultures in terms of milliequivalents CO_2 absorbed and in cell concentrations (calculated from the fluorescence).

The results indicated that the adsorption of Pb, Cu, Cd, and Zn to the filter papers intended as controls could not be typical of all the culture media used. Therefore, the data shown for the concentrations of heavy metals in the cells (Table 3) do not contain a correction for adsorption of the filters, and consequently the results are high. Furthermore, it was apparent that each batch of Instant Ocean® had its own characteristic concentration of trace metals, thereby preventing the comparison of concentrations of heavy metals in the cells from one experiment to another; this would not affect, however, the comparisons between the controls and the elutriate-grown cells in a given experiment. All batches of the G&R medium were prepared with 3.5% NaCl solution from the same stock solutions; therefore the variation in heavy metals was probably a consequence of Instant Ocean® composition.

The sediments and soil samples represented a wide spectrum of physical characteristics with moisture contents ranging from 16% to 79%, and fractions of dry weight passing through a 1.6-mm (1/16-in.) sieve extending from 14% to 97%; some of the sediments from Hood Canal contained many shells and others had none. There were large chemical differences also, but in 14 of the elements analyzed (Al, Si, Ca, Ti, V, Fe, K, Co, Ni, Ga, Se, Sr, Ba, and Hg), the differences among samples were less than an order of magnitude, if the presumption can be made that those noted as "less than" a certain concentration were approximately equal.

Table 3—Analyses of Sediment Extracts (in Instant Ocean®) for Pb, Cd, Cu, and Zn

Sediment	Concentrations (ppm)			
	Pb	Cd	Cu	Zn
A	1.27	0.22	0.06	0.28
B	0.71	0.09	0.10	0.13
C	0.71	0.07	0.07	0.11
D	0.63	0.07	0.04	0.07
E	0.79	0.07	0.09	0.10
F	0.87	0.07	0.07	0.04
G	0.63	0.08	0.07	<0.01
H	0.79	0.07	0.07	0.04
I	0.79	0.07	0.09	0.10
J	0.79	0.09	0.09	0.12
K	0.71	0.07	0.07	0.12
L	0.71	0.07	0.05	0.10
σ	0.08	0.01	0.01	0.02

Table 4—Analyses of Algal Cells for Pb, Cd, Cu, and Zn After 2 Days

Sediment	Concentrations by Mass* (ppm)											
	Controls				Cells in Half-Concentrated Elutriate				Cells in Concentrated Elutriate			
	Pb	Cd	Cu	Zn	Pb	Cd	Cu	Zn	Pb	Cd	Cu	Zn
A	1340 ± 300	340 ± 70	410 ± 50	680 ± 45	10 940 ± 290	350 ± 60	700 ± 40	2400 ± 40	ND	ND	ND	ND
B	1000 ± 250	290 ± 50	830 ± 30	630 ± 35	1070 ± 240	180 ± 50	340 ± 30	620 ± 30	1200 ± 300	390 ± 60	2660 ± 40	670 ± 40
C	730 ± 300	240 ± 80	560 ± 40	2250 ± 45	920 ± 305	270 ± 60	880 ± 40	1500 ± 40	1880 ± 630	3750 ± 1250	2500 ± 830	11 250 ± 830
D	590 ± 240	180 ± 50	270 ± 30	1560 ± 30	450 ± 225	160 ± 45	190 ± 30	1210 ± 30	1650 ± 550	660 ± 110	210 ± 75	1430 ± 75
E	840 ± 240	290 ± 50	370 ± 30	840 ± 30	540 ± 202	200 ± 40	240 ± 25	510 ± 25	550 ± 250	130 ± 20	60 ± 35	510 ± 30
F	437 ± 312	229 ± 62	250 ± 42	646 ± 42	404 ± 288	211 ± 58	211 ± 38	788 ± 38	795 ± 340	250 ± 68	613 ± 45	1545 ± 45
G	1450 ± 370	320 ± 75	390 ± 50	780 ± 50	ND	ND	ND	ND	1490 ± 380	280 ± 75	380 ± 40	630 ± 50
H	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
I	990 ± 265	230 ± 55	285 ± 35	675 ± 35	590 ± 250	180 ± 50	550 ± 35	710 ± 35	1350 ± 360	410 ± 70	2140 ± 50	675 ± 50
J	1071 ± 535	464 ± 107	261 ± 71	1035 ± 71	937 ± 312	229 ± 62	152 ± 41	604 ± 41	750 ± 375	325 ± 75	182 ± 50	725 ± 50
K	480 ± 480	330 ± 90	255 ± 65	1025 ± 60	480 ± 480	450 ± 90	360 ± 60	930 ± 60	480 ± 480	530 ± 105	340 ± 70	990 ± 70
L	930 ± 285	250 ± 60	300 ± 40	720 ± 40	900 ± 240	180 ± 50	160 ± 30	570 ± 30	1370 ± 365	270 ± 50	250 ± 50	760 ± 50

Note: ND = not determined.

*No correction made for metal ions adsorbed to the Millipore® filter.

Table 5—Growth of *Phaeodactylum Tricornutum* in Tests With Sediment Elutriates

Sediment	Controls		Cells in 0.01 mg Hg/1		Cells in Half-Concentrated Elutriate		Cells in Concentrated Elutriate	
	Absorption*	Concentration†	Absorption*	Concentration†	Absorption*	Concentration†	Absorption*	Concentration†
A	0.81	172	0.52	115	0.38	132	?	0
B	0.94	185	0.71	140	1.24	190	0.69	180
C	1.25	205	0.69	142	1.03	195	?	29
D	1.95	245	0.97	170	?	200	?	180
E	1.42	210	0.80	140	?	175	?	120
F	1.16	175	0.80	120	1.13	200	1.04	175
G	0.54	110	0.33	85	0.66	130	0.57	150
H	0.67	140	0.40	ND	?	ND	0.75	172
I	0.91	170	0.39	94	?	140	?	80
J	0.54	110	0.41	80	?	130	?	85
K	0.94	150	0.20	86	?	108	?	50
L	1.28	190	0.63	122	?	165	?	105

Note: ? = electrode malfunction.
ND = not determined.

*Milliequivalents CO_2/liter absorbed.

†Concentration by volume (ppm).

An example of the CO_2 uptake measurements is shown in Fig. 3, with error bars for the three control suspensions. The inhibition by 0.01 mg Hg/1 shown here is typical of most experiments. In terms of total growth achieved in a 48-hr period, however, there was a considerable variation from one experiment to another, with CO_2 absorptions ranging from 0.54 to 1.95 milliequivalents/liter, with an average of 1.03 (Table 5). Part of this variance could be ascribed to differences among the batch cultures used for the inocula. Perhaps a more important factor is the considerable variation in heavy metal composition of the Instant Ocean® from one lot to another. This can be deduced from the concentrations of Pb, Cd, Cu, and Zn in the control cells (Table 3), because they reflect the relative heavy metal concentrations of the 1:12 culture medium used in each experiment. For a given batch of Instant Ocean® there was a characteristic uptake of heavy metals in the control cells; e.g., experiments involving samples A, B, and G were performed with one particular batch of Instant Ocean®, and the control cells in each had a high Pb content.

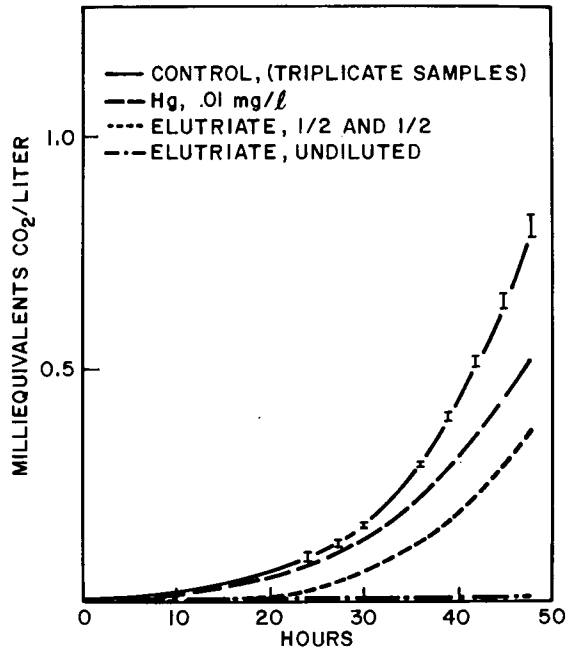

Fig. 3—CO_2 uptake vs time for cultures used in sample A

Cells from suspensions treated with 0.01 mg Hg/1 were not analyzed, but data from earlier studies [15] indicate that these cells would contain approximately 700 ppm Hg. Gaps in the analytical data of Table 4 represent either insufficient sample size or loss of sample.

DISCUSSION

Appraisal of Algal Assay

To estimate the ecological consequences of dredge spoils disposal from the results obtained with one organism, or class of organisms, would be risky. Therefore no claim will be made that the algal approach is to be preferred above all others; rather, this discussion will stress its relative advantages and disadvantages. Some of the advantages are:

1. The time required is short.

2. Good correlations have been obtained with other assays that rank the relative toxicities of compounds.

3. The algae are avid scavengers of heavy metals and are therefore useful in other matters related to this problem. For example, appreciable concentrations of heavy metals can be accumulated by the cells without entirely depleting the reservoir of trace metals in the medium.

A total review of the information gained from this study would be too lengthy, but several points deserve mention. First, the test is sensitive to numerous ions or compounds at concentrations considerably less than 1 ppm, and at these low concentrations any adsorptive effects can be overwhelming. It is necessary, therefore, to use freshly prepared solutions and also to use as culture vessels large enough to provide a low surface per volume ratio. For this reason the 500 ml Erlenmeyer flask was adopted; under nutrient-limited culture conditions there was as much growth in this flask as in 1000 ml, whereas growth in a 250 ml size was considerably less. Second, regarding the advantages of continuous growth measurements (viz, pH) over one measurement made at the end of a fixed time period, the results were gratifying but not necessarily essential. They showed, however, that when *Phaeodactylum tricornutum* was cultured under the conditions described there was a linear relationship between the log of milliequivalents of CO_2 absorbed vs time between 36 and 48 hr. Therefore, continuous measurements over this period are not required to determine a growth rate, and a good estimate can be made by two measurements during that time. Third, the combination type of electrodes used for the pH measurements are subject to interference from the light and from compounds released from the samples during elutriation. If the electrodes are conditioned overnight in a culture medium of the type used in the growth study, and if they are illuminated at the same time, the "drift" of the electrodes is sufficiently controlled, but no antidote has been sought to negate the effects of the soil elutriates. The effect of light on the electrode can be completely nullified by substituting a calomel electrode for the Ag/AgCl reference, but the release of Hg from the calomel is sufficient to affect the growth of the culture. Estimates of cell number by measurements of the fluorescence of the cultures are only approximate for a variety of reasons, but they take little time to make and they give a qualitative measure that is generally satisfactory. Subsequent experimentation has shown that the results can be made more quantitative with little change in the procedures used here [16]. By the addition of the herbicide DCMU (see Fig. 4) to the cell suspension, the photosynthesis of the cells is stopped, and the resulting enhanced fluorescence is a direct reflection of the chlorophyll present; for cells of different sizes but identical chlorophyll content, the fluorescence is the same.

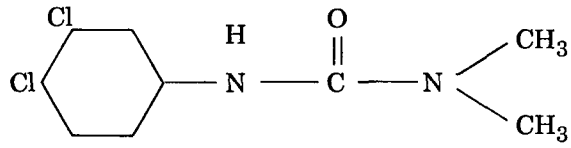

Fig. 4—Structure of DCMU

Relationship Between Heavy Metals in Test Samples and in Algal Cells

It was not feasible to analyze the algal cells for all the metals listed in Table 2; therefore the study was limited to Pb, Cu, Zn, and Cd. Though Cd had not been included in the x-ray fluorescence analyses of the sediment and soil samples, its concentration in the algal cells was determined because of its relevance as a pollutant. There was little relationship between the concentration of Pb, Cu, and Zn in the sediments and the resulting concentration in the cells. For example, sample D promoted increased Pb concentration of cells grown in the concentrated elutriate, although it was one of the lowest in Pb content itself; sample I promoted Cu uptake despite being only intermediate in Cu content. On the other hand, sample E probably was an efficient scavenger for metals in the process of elutriation because cells grown in the corresponding culture were particularly low in Cd, Cu, and Zn. This particular sample consisted of fine particles whose high surface area may have been a factor in the exchange process. Sample C was most prominent in fostering an increase in the concentration of all four metals studied, yet it was second lowest in Cu, third lowest in Zn, and second lowest in Pb. The most apparent instance of a correlation between sample metal and cell metal occurred with A; this sample had the highest Pb content by far, and so did the cells grown in the half-concentrated elutriate. There was no growth with the concentrated elutriate culture, perhaps because of the Pb uptake by the cells.

Chemical speciation in the sediment or soil sample undoubtedly affects the rate of release and subsequent effects on the uptake by algae. It is possible that samples that bind a given metal loosely will not accumulate large concentrations of it, but the amount present may be more labile than that contained in a sediment containing a higher concentration. Complexation of metals is recognized as an important factor in their effect on aquatic organisms. Concentrations of Cu in Clear Lake, Calif., were high enough to be toxic except that they were complexed by naturally occurring substances that reduced the activity of the copper [17]. In another study [18] the degree of chelation of copper could be estimated by a bioassay involving the growth of *Thalassiosira pseudonana*.

The effect of trace metals in the filter papers used for the collection of the algal cells on the analytical results was considered. Each filter weighed approximately 100 mg and contained roughly 0.35 ppm Pb, 4.8 ppm Cu, and 3.1 ppm Zn [19], but its contribution to the total amounts analyzed would not be great enough to dominate the results. Approximately 80% of the metals accounted for in the analyses could be attributed to the cells, the remainder being the metals present in the filter paper at the time of manufacture or subsequently adsorbed from the culture medium/Instant Ocean® solution.

Interpretations of Algal Growth and Metal Accumulation Data

Most of this commentary concerns the results with the concentrated elutriate suspensions because they are considered more pertinent to the dredge spoils problem. In estimating the pollution hazard of many sediments there is room for debate about the proportions of sediment and water used in the elutriation; in this study the ratio was 1:7, whereas the Standard Elutriate Test specifies 1:4. More recent work suggests that 1:20 might be a better proportion, because there is less of a handling problem and this ratio of solids to water is often encountered in hopper dredges.*

Of primary interest was whether a rationale could be offered for the toxicities of the sediments that inhibited the growth of algae. In most cases this was possible; for example, sediments A and C were taken from dump sites for various materials. Area A had been used for the disposal of Otto fuel, but the greasy character and high Pb content of the sediment suggested that perhaps other waste materials had been disposed of there as well. In the algal assay there was no growth in the concentrated elutriate of A but appreciable growth in the half-concentrated elutriate despite an enormous accumulation of Pb by the cells. It is possible that the toxicity of the concentrated elutriate was more the consequence of the oil in the elutriate than Pb. In the case of area C, the sediment toxicity could be related logically to its use as a dump site for RDX, TNT, and scrap metal for some time. Also the samples from both A and C had the greatest effect on the initial pH of the algal suspensions, reducing it by 1.38 and 2.43 units, respectively.

Samples E and I caused about 50% growth inhibition, which was interesting in that they were sediments from small freshwater lakes in which fish occur. No reason can be suggested for the toxicity of E insofar as metals are concerned, because the cells grown in the elutriate had generally lower concentrations of the four metals analyzed than did the controls. With I there was an appreciable buildup of Cu, which may or may not have been the controlling factor. Sample D, which was the site of an old pier, caused about 25% growth inhibition, perhaps because of the increased Pb and Cd in the algal cells. Samples K and L were from the Hood Canal and both were somewhat toxic. K is located in front of a private beach where a boat is often moored, and it is also the site of a water-quality monitor, and L represents Marginal Wharf where large ships can be moored. The effects of the elutriates from these sediments on the composition of the algal cells were not significant; therefore, their effects on the growth may have been because of some element other than Pb, Cu, Cd, or Zn, or some organic compounds. Sample B was taken from the KB wharf, which has been used for mooring ships of approximately 30 m (100 ft) in length, and although it promoted a buildup of Cu in the algal cells, it was not toxic.

In contrast to the toxic nature of the sediments from the freshwater lakes (E and I), the samples from rapidly flowing shallow streams (G and H) promoted an increase in the algal growth rate. Unfortunately, no analyses could be made of the cells grown in the elutriate of H because of an accidental loss of the sample, but the composition of the cells obtained with G was no different than the controls.

*G. Fred Lee, University of Texas at Dallas, private communication.

One major impression gained from studying these results is that great differences can occur in sediments that are relatively close to each other, and therefore when a judgment must be made about a certain site being safe for dredging, a large number of samples should be pooled and examined. This variability is also evident in an examination of the sediments taken from the Hood Canal, where many contain great amounts of shells and others contain none.

The results obtained suggest that the Algal Toxicity Test used in this study can be a useful tool in classifying sediments according to their toxicity; it should be compared with toxicity tests involving other organisms, including fish, to determine how accurately it can predict whether sediments are pollution hazards. Further study is needed to make it an even more reliable test procedure; work at NRL has indicated that a totally synthetic seawater, made with CP chemicals, when made in volumes of several hundred liters would be useful in repetitive tests where direct comparisons from one test to another are sought. Also, a continuous culture device has been assembled to provide cells of a constant composition, which should greatly reduce the variability resulting from the use of batch cultures.

Phaeodactylum tricornutum was the only organism used in this particular study because of the background of experience developed in related studies. The use of other organisms chosen for their peculiar tolerances or susceptibilities would also provide useful information.

Despite the boundaries of the interpretations permitted with toxicity studies involving one or several organisms, the information gained is considered more applicable than that derived from multiple chemical analyses. This study showed that there was almost no correlation between the heavy metal content of the sediments and the composition of algal cells grown in their elutriates. Even exhaustive analyses of the elutriates themselves, for potential inorganic and organic hazards, would often not be interpretable in absolute terms.

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